

Amendments to the Specification

Please amend the specification as follows:

Please amend the paragraph on page 4, line 16, to page 5, line 4, as follows:

Accordingly, the present invention relates to a peptide which has at least the following amino acid sequence **(SEQ ID NO: 1)**

| | |
|--|----|
| Ser-Cys-Asn-Thr-Ala-Thr-Cys-Met-Thr-His- | 10 |
| Arg-Leu-Val-Gly-Leu-Leu-Ser-Arg-Ser-Gly- | 20 |
| Ser-Met-Val-Arg-Ser-Asn-Leu-Leu-Pro-Thr- | 30 |
| Lys-Met-Gly-Phe-Lys-Val-Phe-Gly | 38 |

, or an amino acid sequence where a part of amino acids are deleted, substituted or added, and has the following characteristics: (1) being expressed in central nervous system, (2) strongly acting on calcitonin receptors and (3) promoting a cAMP productivity of cells, more preferably, (4) incorporating sodium ion in a concentration-depending manner, (5) suppressing the uptake of calcium ion, and (6) suppressing cell proliferation.

Please amend the paragraph on page 6, lines 17-18, as follows:

Fig. 1 schematically shows the structure of CRSP of the present invention **(SEQ ID NO: 1)**.

Please amend the paragraph on page 6, lines 19-24, as follows:

Fig. 2 shows comparisons of the amino acid sequence of CRSP (pCRSP, **SEQ ID NO: 1**) of the present invention with amino acid sequences of pig calcitonin gene-related peptide (pCGRP-I, **SEQ ID NO: 23**), human calcitonin gene-related peptide I (hCGRP-I, **SEQ ID NO: 24**), human calcitonin gene-related peptide II (hCGRP-II, **SEQ ID NO: 25**),

human amylin (hAmylin, SEQ ID NO: 26), pig calcitonin (pCT, SEQ ID NO: 27) and human adrenomedullin (hAM, SEQ ID NO: 28).

Please amend the paragraph on page 11, lines 9-11, as follows:

Fig. 17 shows a nucleotide sequence of CRSP gene (SEQ ID NO: 5). The underlined parts show exons. Beneath the underlined parts, the amino acid sequences coded by CRSP gene are is shown (SEQ ID NO: 4).

Please amend the paragraph on page 11, lines 12-13, as follows:

Fig. 18 shows the first half part (bases of from 1 to 3840 of SEQ ID NO: 15) of the nucleotide sequence of CRSP-2 gene.

Please amend the paragraph on page 11, lines 14-17, as follows:

Fig. 19 shows the second half part (bases of from 3841 to 7673 of SEQ ID NO: 15) of the nucleotide sequence of CRSP-2 gene. The underlined parts show exons. Beneath the underlined parts, the amino acid sequences coded by CRSP-2 gene is are shown (SEQ ID NO: 14).

Please amend the paragraph on page 11, lines 18-21, as follows:

Fig. 20 shows a cDNA nucleotide sequence of CRSP-2 (SEQ ID NO: 13). In Fig. 20, an area enclosed by a solid line is a mature peptide (SEQ ID NO: 14) and the part in gray color shows glycine which is presumed to be converted to an amide.

Please amend the paragraph on page 11, lines 22-25, as follows:

Fig. 21 shows a cDNA nucleotide sequence of CRSP-3 (**SEQ ID NO: 17**). In Fig. 21, the area enclosed by a solid line is a mature peptide (**SEQ ID NO: 18**) and the part in gray color shows glycine which is presumed to be converted to an amide.

Please amend the paragraph on page 12, lines 1-6, as follows:

Fig. 22 shows a DNA nucleotide sequence of CT-2 (**SEQ ID NO: 20**). In Fig. 22, an area enclosed by a solid line is a mature peptide (**SEQ ID NO: 21**) and the part in gray color shows glycine which is presumed to be converted to an amide. Glutamine located at the N-terminal side of mature substance in Fig. 22 is presumed to be converted to pyroglutamic acid.

Please amend the paragraph on page 12, lines 7-8, as follows:

Fig. 23 shows a drawing where amino acids of CRSP, CGRP, AM, CT-2 and CT are compared (**SEQ ID NOS 12, 16, 1, 23, 29, 27, and 19, respectively in order of appearance**).

Please amend the paragraph on page 12, line 24, to page 13, line 16, as follows:

The present inventors have purified two kinds of novel physiologically active peptides from an extract of porcine brain using a cAMP production of renal epithelial cells as an index. When the amino acid sequence of the resulting peptides is analyzed, it has been found that they are a peptide (hereinafter, it will be referred to as CRSP) comprising the following 38 amino acids (**SEQ ID NO: 1**)

| | |
|---|----|
| Ser-Cys-Asn-Thr-Ala-Thr-Cys-Met-Thr-His- | 10 |
| Arg-Leu-Val-Gly-Leu-Leu-Ser-Arg-Ser-Gly- | 20 |
| Ser-Met-Val-Arg-Ser-Asn-Leu-Leu-Pro-Thr- | 30 |
| Lys-Met-Gly-Phe-Lys-Val-Phe-Gly-NH ₂ | 38 |

and a peptide (hereinafter, it will be referred to as CRSP-G) comprising the following 39 amino acids **(SEQ ID NO: 2)** where glycine is bonded to a C-terminal of the above described peptide.

| | |
|--|----|
| Ser-Cys-Asn-Thr-Ala-Thr-Cys-Met-Thr-His- | 10 |
| Arg-Leu-Val-Gly-Leu-Leu-Ser-Arg-Ser-Gly- | 20 |
| Ser-Met-Val-Arg-Ser-Asn-Leu-Leu-Pro-Thr- | 30 |
| Lys-Met-Gly-Phe-Lys-Val-Phe-Gly-Gly-OH | 39 |

Please amend the paragraph on page 13, line 25, to page 14, line 6, as follows:

On the basis of the amino acid sequence, the primer used was

TG (C/T) AA (C/T) AC (A/C/G/T) GC (A/C/G/T) AC (A/C/G/T) TG (C/ T)ATGAC
(SEQ ID NO: 31)

at the N-terminal side and was

CC(A/G)AA(A/C/G/T)AC(C/T)TT(A/G)AA(A/C/G/T)CCCATA **(SEQ ID NO: 32)** at the C-terminal side.

Please amend the paragraph on page 14, lines 7-23, as follows:

A base sequence of the resulting gene is shown in SEQ ID NO: 3 of Sequence Listing and an amino acid sequence coded thereby is shown as follows **(SEQ ID NO: 4)**.

| | |
|--|----|
| Met-Gly-Phe-Trp-Lys-Phe-Pro-Pro-Phe-Leu- | 10 |
| Val-Leu-Ser-Ile-Leu-Val-Leu-Tyr-Gln-Ala- | 20 |
| Gly-Met-Phe-His-Thr-Ala-Pro-Met-Arg-Ser- | 30 |
| Ala-Phe-Gly-Ser-Pro-Phe-Asp-Pro-Ala-Thr- | 40 |

| | |
|---|-----|
| Leu-Ser-Glu-Glu-Glu-Ser-Arg-Leu-Leu-Leu- | 50 |
| Ala-Ala-Met-Val-Asn-Asp-Tyr-Glu-Gln-Met- | 60 |
| Lys-Ala-Arg-Glu-Met-Gln-Lys-Gln-Arg-Ala- | 70 |
| Gln-Gly-Ser-Gly-Ile-Ser-Val-Gln-Lys-Arg- | 80 |
| <u>Ser-Cys-Asn-Thr-Ala-Thr-Cys-Met-Thr-His-</u> | 90 |
| <u>Arg-Leu-Val-Gly-Leu-Leu-Ser-Arg-Ser-Gly-</u> | 100 |
| <u>Ser-Met-Val-Arg-Ser-Asn-Leu-Leu-Pro-Thr-</u> | 110 |
| <u>Lys-Met-Gly-Phe-Lys-Val-Phe-Gly-Gly-Arg-</u> | 120 |
| Arg-Arg-Asn-Phe-Trp-Ile | 126 |

(In the formula, the underlined 81st to 118th ones are CRSP.)

Please amend the paragraph on page 15, lines 4-7, as follows:

An amino acid sequence of bovine CRSP represented by one-letter codes for amino acids is as follows.

ACNTATCMTHRLAGWLSRSG

SMVRSNLLPTKMGFKIFNGP-OH **(SEQ ID NO: 6)**

Please amend the paragraph on page 15, lines 8-13, as follows:

When it is represented by three-letter codes for amino acids, it is as follows **(SEQ ID NO: 6)**.

| | |
|--|----|
| Ala-Cys-Asn-Thr-Ala-Thr-Cys-Met-Thr-His- | 10 |
| Arg-Leu-Ala-Gly-Trp-Leu-Ser-Arg-Ser-Gly- | 20 |
| Ser-Met-Val-Arg-Ser-Asn-Leu-Leu-Pro-Thr- | 30 |

Lys-Met-Gly-Phe-Lys-Ile-Phe-Asn-Gly-Pro-OH

40

Please amend the paragraph on page 15, lines 14-17, as follows:

An amino acid sequence of canine CRSP represented by one-letter codes for amino acids is as follows.

SCNSATCVAHWLGGLSRAG

SVANTNLLPTSMGFKVYN-OH **(SEQ ID NO: 9)**

Please amend the paragraph on page 15, lines 18-23, as follows:

When it is represented by three-letter codes for amino acids, it is as follows **(SEQ ID NO: 9)**:

| | |
|--|----|
| Ser-Cys-Asn-Ser-Ala-Thr-Cys-Val-Ala-His- | 10 |
| Trp-Leu-Gly-Gly-Leu-Leu-Ser-Arg-Ala-Gly- | 20 |
| Ser-Val-Ala-Asn-Thr-Asn-Leu-Leu-Pro-Thr- | 30 |
| Ser-Met-Gly-Phe-Lys-Val-Tyr-Asn-OH | 38 |

Please amend the paragraph on page 16, lines 19-24, as follows:

An amino acid sequence of CRSP-2 as represented by three-letter codes for amino acids is as follows **(SEQ ID NO: 12)**.

| | |
|--|----|
| Ser-Cys-Asn-Thr-Ala-Ser-Cys-Val-Thr-His- | 10 |
| Lys-Met-Thr-Gly-Trp-Leu-Ser-Arg-Ser-Gly- | 20 |

| | |
|---|----|
| Ser-Val-Ala-Lys-Asn-Asn-Phe-Met-Pro-Thr- | 30 |
| Asn-Val-Asp-Ser-Lys-Ile-Leu-NH ₂ | 37 |

Please amend the paragraph on page 16, line 25, to page 17, line 5, as follows:

An amino acid sequence of CRSP-3 as represented by three-letter codes for amino acids is as follows **(SEQ ID NO: 16)**.

| | |
|---|----|
| Ser-Cys-Asn-Thr-Ala-Ile-Cys-Val-Thr-His- | 10 |
| Lys-Met-Ala-Gly-Trp-Leu-Ser-Arg-Ser-Gly- | 20 |
| Ser-Val-Val-Lys-Asn-Asn-Phe-Met-Pro-Ile- | 30 |
| Asn-Met-Gly-Ser-Lys-Val-Leu-NH ₂ | 37 |

Please amend the paragraph on page 17, lines 6-11, as follows:

An amino acid sequence of CT-2 as represented by three-letter codes for amino acids is as follows **(SEQ ID NO: 19)**.

| | |
|--|----|
| Glu-Cys-Asn-Asn-Leu-Ser-Thr-Cys-Val-Leu- | 10 |
| Gly-Thr-Tyr-Thr-Trp-Asp-Val-Asn-Lys-Phe- | 20 |
| Tyr-Ala-Phe-Pro-Leu-Thr-Thr-Thr-Gly-Ile- | 30 |
| Arg-Val-Ser-NH ₂ | 33 |

Please amend the paragraph on page 30, line 17, to page 31, line 11, as follows:

The peptides of the present invention have characteristics of: (1) being expressed in central nervous systems, (2) strongly acting on calcitonin receptor, (3) promoting a cAMP productivity of cells, and relate to the peptides which have at least the following amino acid sequence **(SEQ ID NO: 1)**

| | |
|--|----|
| Ser-Cys-Asn-Thr-Ala-Thr-Cys-Met-Thr-His- | 10 |
| Arg-Leu-Val-Gly-Leu-Leu-Ser-Arg-Ser-Gly- | 20 |
| Ser-Met-Val-Arg-Ser-Asn-Leu-Leu-Pro-Thr- | 30 |
| Lys-Met-Gly-Phe-Lys-Val-Phe-Gly | 38 |

or an amino acid sequence where a part of the amino acids are deleted, substituted or added. The peptides of the present invention include amide derivatives, ester derivatives and derivatives of functional group of side chain of each amino acid such as amino group of lysine and hydroxyl group of serine provided that the above described amino acid sequence is comprised. More preferable peptides of the present invention further have the characteristics of: (4) incorporating sodium ion concentration-dependently, (5) suppressing the incorporation of calcium ion, (6) suppressing the cell proliferation.

Please amend the paragraph on page 39, lines 9-15, as follows:

An amino acid sequence was determined by an Edman method using an automatic amino acid sequencer. Firstly, 5 pmol of the pure specimen was analyzed by the amino acid sequencer from its N-terminal and the following was determined.

Ser-Xaa-Asn-Thr-Ala-Thr-Xaa-Met-Thr-His-Arg-Leu-Val-Gly-Leu-Leu-Ser-Arg-Ser-Gly-Ser-Met-Val **(SEQ ID NO: 33)**

Please amend the paragraph on page 39, line 16, to page 40, line 5, as follows:

After that, 10 pmol of the pure specimen was subjected a trypsin degradation and subjected to a concentration-gradient elution by acetonitrile using a reversed phase HPLC (C₁₈ 218TP5215, Vydac, 2.1 x 150 mm; solution A: water : acetonitrile : 10% trifluoroacetic acid = 90:10:1; solution B: water : acetonitrile : 10% trifluoroacetic acid = 40:60:1; flow rate: 0.2 ml/min) . The resulting peaks were analyzed by the amino acid sequencer whereupon the following six sequences were able to be obtained.

Ser-Xaa-Asn-Thr-Ala-Thr-Xaa-Met-Thr-His-Arg **(SEQ ID NO: 34)**,

Leu-Val-Gly-Leu-Leu-Ser-Arg (**SEQ ID NO: 35**),

Ser-Gly-Ser-Met-Val-Arg (**SEQ ID NO: 36**),

Ser-Asn-Leu-Leu-Pro-Thr-Lys (**SEQ ID NO: 37**),

Met-Gly-Phe-Lys (**SEQ ID NO: 38**) and

Val-Phe

Please amend the paragraph on page 40, lines 6-12, as follows:

As a result of comparison of those sequences with the databases, it was concluded that the present peptide has a homology to a calcitonin gene-related peptide and has the following sequence (**SEQ ID NO: 30**).

Ser-Cys-Asn-Thr-Ala-Thr-Cys-Met-Thr-His-Arg-Leu-Val-Gly-Leu-Leu-Ser-Arg-Ser-Gly-Ser-Met-Val-Arg-Ser-Asn-Leu-Leu-Pro-Thr-Lys-Met-Gly-Phe-Lys-Val-Phe-NH₂

Please amend the paragraph on page 40, line 13, to page 41, line 1, as follows:

In the meanwhile, its molecular weight was measured using a mass spectrometer and the result was 4, 130.6 ± 0.7 Da. This was different from 4, 042 Da presumed from the amino acid sequence to an extent of about 89 Da but the difference is presumed to be due to oxidation of two methionines (2 x 16 Da) and the presence of glycine (57 Da) at C-terminal which was not able to be measured by the amino acid sequencer. Finally, from the next Example 3, it was found to be as follows which was identical with those presumptions.

Ser-Cys-Asn-Thr-Ala-Thr-Cys-Met-Thr-His-Arg-Leu-Val-

Gly-Leu-Leu-Ser-Arg-Ser-Gly-Ser-Met-Val-Arg-Ser-Asn-

Leu-Leu-Pro-Thr-Lys-Met-Gly-Phe-Lys-Val-Phe-Gly-NH₂ (**SEQ ID NO: 1**)

(A disulfide bond is formed between the second Cys and the seventh Cys.)

Please amend the paragraph on page 41, lines 7-15, as follows:

A probe was prepared by formation of a synthetic primer using

(TG (C/T) AA (C/T) AC (A/C/G/T) GC (A/C/G/T) AC (A/C/G/T) TG (C /T)ATGAC)

(SEQ ID NO: 31)

of the N-terminal side and

(CC (A/G) AA (A/C/G/T) AC (C/T) TT (A/G) AA (A/C/G/T) CCCATA)

(SEQ ID NO: 32)

of the C-terminal side on the basis of the amino acid sequence of Example 2 and by amplification using a PCR where pig gene was used as a template.

Please amend the paragraph on page 59, line 13, to page 60, line 1, as follows:

Mature amino acid sequences of CRSP-2, CRSP-3 and CT-2 are presumed to be as follows.

| | | |
|---------------------------|---|----|
| CRSP-2: | Ser-Cys-Asn-Thr-Ala-Ser-Cys-Val-Thr-His | 10 |
| <u>(SEQ ID NO:</u> | Lys-Met-Thr-Gly-Trp-Leu-Ser-Arg-Ser-Gly | 20 |
| <u>12)</u> | | |
| | Ser-Val-Ala-Lys-Asn-Asn-Phe-Met-Pro-Thr | 30 |
| | Asn-Val-Asp-Ser-Lys-Ile-Leu-NH ₂ | 37 |
| CRSP-3: | Ser-Cys-Asn-Thr-Ala-Ile-Cys-Val-Thr-His | 10 |
| <u>(SEQ ID NO:</u> | Lys-Met-Ala-Gly-Trp-Leu-Ser-Arg-Ser-Gly | 20 |
| <u>16)</u> | | |
| | Ser-Val-Val-Lys-Asn-Asn-Phe-Met-Pro-Ile | 30 |
| | Asn-Met-Gly-Ser-Lys-Val-Leu-NH ₂ | 37 |
| CT-2: | pGlu-Cys-Asn-Asn-Leu-Ser-Thr-Cys-Val-Leu | 10 |

| | | |
|-------------------------------|---|----|
| <u>(SEQ ID NO: 19)</u> | Gly-Thr-Tyr-Thr-Trp-Asp-Val-Asn-Lys-Phe | 20 |
| | Tyr-Ala-Phe-Pro-Leu-Thr-Thr-Thr-Gly-Ile | 30 |
| | Arg-Val-Ser-NH ₂ | 33 |

Please amend the paragraph on page 61, line 24, to page 62, line 9, as follows:

A PCR where pig hypothalamus cDNA (corresponding to 20 ng of mRNA) was a template was carried out using primers (CRSP-3: GCCCAGCTTACGTCTCCTTT **(SEQ ID NO: 39)** and TCAGGTAAGTCAATGATTT **(SEQ ID NO: 40)**; CT-2: AGCAGCTTTGATTCTGCCAC **(SEQ ID NO: 41)** and ACCTCCTCTCTGATATTCCA **(SEQ ID NO: 42)**) and Pyrobest DNA polymerase of Takara Shuzo for 30 cycles each comprising at 94°C for 15 seconds, at 55°C for 15 seconds and at 72°C for 1 minute. The amplified DNA was subjected to an agarose gel electrophoresis, bands stained with ethidium bromide were recovered from agarose gel and subcloned into pBluescript II of Stratagene and a nucleotide sequence was determined by a Sanger method.

Please amend the paragraph on page 62, line 18, to page 63, line 7, as follows:

A template cDNA was prepared using total RNA (4 µg) of each tissue of pig, oligo dT primer and a Revertra Ace reverse transcriptase kit of Toyobo and 1/40 thereof was used for RT-PCR. The PCR for amplification of each genetic cDNA was conducted using rTaq polymerase of Toyobo in 30 cycles each comprising at 94°C for 15 seconds, at 60°C for 15 seconds and at 72°C for 1 minute and, with regard to the primer, the following sequences were used.

CRSP: CTCTCTGAGGAGGAATCACG **(SEQ ID NO: 43)** and
 GAGTTCAGAGTCATAGTAACC **(SEQ ID NO: 44)**

CRSP-2: CTCACAGAGGAGGAAGTCTC **(SEQ ID NO: 45)** and
 TAGAGTTCAGTTCCTTGGTG **(SEQ ID NO: 46)**

CRSP-3: AGCAGCTTTGATTCTGCCAC (SEQ ID NO: 41) and
TGCAGTGAAAGCAACTTGAG (SEQ ID NO: 47)

CT-2: AGCAGCTTTGATTCTGCCAC (SEQ ID NO: 41) and
ACCTCCTCTCTGATATTCCA (SEQ ID NO: 42)

CT: GCCACTCAGTGAGAAGGAAG (SEQ ID NO: 48) and
TGAGGCATGAGGGATGAAGC (SEQ ID NO: 49)

CGRP: GCCACTCAGTGAGAAGGAAG (SEQ ID NO: 48) and
TCACCTTACATGTGTCCCCA (SEQ ID NO: 50)

GAPDH: TCACTGCCACCCAGAAGACT (SEQ ID NO: 51) and
AGTGGTCGTTGAGGGCAATG (SEQ ID NO: 52)